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The Effect of Adding Coconut Sugar on Quality of Goat Milk Whey Kefir Seasoning from Physical, Chemical and Microbiological Qualities

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Abstract**—** *The research aims to determine the effect of adding the best coconut sugar to improve the quality of the end product which includes pH, total acidity, protein content, Total Plate Count (TPC), mold and yeast. The used materials ware whey kefir obtained from the separation of the solid (curd) and liquid (whey) parts of goat's milk kefir products with supporting ingredients such as kefir grains, spices (ginger, lime leaves, Illicium verum and cardamom), salt and coconut sugar concentrations of 20, 30, 40 and 50%. The research method used was a laboratory experiment with a Completely Randomized Design consisting of 4 addition the concentration of coconut sugar 20, 30, 40, and 50% in whey kefir seasoning with 6 replications. The data were analyzed using Analysis of Variance (ANOVA) and if there were significant influence would test by Duncan's Multiple Range Test Method (DMRT). The result showed that there was a highly significant difference effect (P<0.01) in the addition of coconut sugar to pH, total acidity, protein content, TPC, mold and yeast. The addition of the concentration of coconut sugar solution of 50% is the best treatment with a pH value of 4.36, total acidity of 1.02%, the protein content of 1.13%, Total Plate Count (TPC) of 3.45 log cfu/ml, mold and yeast of 2.76 log cfu/ml.*

Keywords**—** *Seasoning whey kefir, coconut sugar.*

I. INTRODUCTION

Currently, many food products have been developed that combine nutrition and health functions, commonly referred to as functional food products. An example of a functional food product is kefir. Julianto, Rossi and Yusmarini (2016) stated that in the fermentation process, kefir might be separated into two fractions curd and whey due to production errors such as adding too much kefir grain percentage, temperature fluctuation, RH of the fermentation room, and the fermentation process which is too long. This might result in kefir with a more acidic taste, lower pH, and more liquid kefir products as well as producing a separate kefir texture between curd and whey. Thus, it is organoleptically less favored by consumers.

Whey kefir contains functional proteins such as α-lactalbumin, β lactoglobulin, lactoferrin, lactoperoxidase, immunoglobulin, and serum albumin (Jaya, Purwadi and Widodo, 2017). The components of whey kefir include 0.76% protein, 0.09% fat, and 0.53% minerals (USDA, 2009). Besides, whey kefir also contains flavor components namely lactic acid, diacetyl, acetaldehyde, and acetic acid (Magalhaes, Dragone, Pereira, Oliveira, Domingues, Teixeira, Silvia, and Schwan, 2011). Thus, whey kefir reprocessing can be utilized in making seasonings.

Processing whey kefir into seasoning with the addition of other ingredients such as coconut sugar and other supporting ingredients such as spices (ginger, lime leaves, *Illicium verum*, and cardamom) and salt can produce flavor and are useful as a flavor and aroma enhancer of a food product (Khezri, Sayedsaleh, Hasanpour, Dastras and Dhehghan, 2016).

A study on the use of whey kefir in the seasoning with the addition of coconut sugar is still rarely done and is a new thing to be developed. Thus, the findings on whey kefir seasoning might be able to potentially open up new opportunities in market segmentation that might increase the variety and quality of livestock products. Whey kefir seasoning products are products that prioritize natural ingredients that are certainly safe for health and have a high selling value.

II. MATERIALS AND METHODS

A. Materials

The materials used in this study were whey kefir obtained from the separation of the solid (curd) and liquid (whey) parts of goat's milk kefir and additional supporting ingredients such as kefir grains, (ginger 3.0% (w/v), lime leaves 3.0% (w/v), *Illicium verum* 3.0% (w/v) and cardamom 3.0% (w/v)), salt 2.0% (w/v) and coconut sugar. The instruments used to make whey kefir seasoning included a stove, a pan, a measuring cup, a scale, and a stirrer.

Whey kefir seasoning was made with a mixture of spices (ginger, lime leaves, *Illicium verum* and cardamom) and salt, as well as a coconut sugar solution according to the treatment, 20, 30, 40 and 50%. Meanwhile, the heating process was carried out at 70°C for 30 minutes.

B. Analyzied Procedure

The pH was tested using a pH meter (Hidayat, Kusrahayu and Mulyani, 2013). The pH meter was calibrated in advance by using a buffer of pH 4 and pH 7. The electrode into the sample to be tested was inserted at room temperature. After that, the numbers printed on the pH meter screen after a constant state was recorded.

Total acid was tested using titration (Sinurat, Ekowati and Sunardi, 2018). A sample of 10ml was taken and placed in the Erlenmeyer for titration. 1 drop of 1% PP indicator solution

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was added and drained with 0.1 N of NaOH until a constant pink color was seen.

Protein content was tested utilizing formal titration (Soenarno, Polli, Febrisiantosa and Hanifah, 2013). 10 ml of the sample was put into the Erlenmeyer flask. Then, 20 ml of aquadest, a few drops of 1% phenolphthalein, and 0.4 ml of saturated potassium oxalate were added and let alone for 2 minutes. 0.1 N of NaOH solution was titrated until a pink color appeared. Then, 2 ml of 40% formaldehyde was added followed by titration of 0.1N of NaOH until the solution turned pink. The amount of NaOH used as the titration volume of the sample was noted. At last, a blank solution was made in the same way using aquadest as a substitute for the sample.

The testing procedure of TPC (Total Plate Count) was conducted using Plate Count Agar (PCA) media by pour plate (Yunita, Hendrawan and Yulianingsih, 2015). 10^{-1} dilution was carried out by transferring 1 ml of milk into 9 ml of 0.1% Buffered Peptone Water solution. The same way was also done on 10^{-2} to 10^{-5} test tubes. 1 ml of 10^{-3},10^{-4} and 10^{-5} suspension was put into each petri dish. 10-15ml of PCA (Plate Count Agar) was poured into a petri dish filled with suspension and then each of them was homogenized using the number 8. After that, solidify was allowed at room temperature before being incubated for 18-24 hours at 35-37°C. After incubation, the total bacteria in the dish were calculated.

Mold and yeast was done using Potato Dextrose Agar (PDA) as a pour plate (Rismana, 2016; Atma, 2016). The microbial contamination rate of mold and yeast was determined by preparing 3 tubes filled with 9ml of agar distilled water. From the results of the homogenization, 1 ml of 10^{-1} dilution pipettes into the agar distilled water were as follows. First, a 10^{-2} dilution was obtained and then made up to a 10^{-4} dilution. Then, 15-20 ml of liquid PDA media (temperature 45°C) was put into a petri dish and allowed to solidify/ freeze. Then, 1 ml of the diluted sample was put into a petri dish that already contained solid PDA media. After that, the sample was immediately shaken and rotated until the suspension was evenly distributed. Incubation was carried out at 25°C for 3-5 days.

Data were analyzed using Analysis of Variance (ANOVA) and if there is a significant difference, it might be tested by the Duncan's Multiple Range Test (DMRT) method.

III. Result and Discussion

The Effect of Coconut Sugar Solution on the pH test, Total Acid, and Protein Content on Whey Kefir Seasoning

The results of this study on the pH test, total acid, and protein content of the whey kefir seasoning without the addition of coconut sugar solution (P0), and whey kefir seasoning with the addition of coconut sugar solution 20% (P0), 30% (P2), 40% (P3) 50% (P4) are presented in Table I.

The pH of Whey Kefir Seasoning

The analysis of variance showed that the addition of different concentrations of coconut sugar solution gave a very significant difference ($P<0.01$) on the pH of the whey kefir seasoning. The average value in Table I indicates that the average pH value of the whey kefir seasoning increased with the addition of the concentration of coconut sugar solution. The lowest pH average value was at P0, 4.22 ± 0.01, without the addition of the coconut sugar solution concentration, and the highest pH average value was at P4, 4.36 ± 0.01, with the addition of the coconut sugar solution concentration of 50%. The increase in the average pH value of whey kefir was due to the higher concentration of coconut sugar solution added might cause a decrease in the activity of bacteria that played a role in the formation of lactic acid. The decrease in bacterial activity was thought to be due to the addition of the concentration of coconut sugar solution which might cause bacterial cells to lose water for their life activities. Thus, bacterial growth was inhibited and resulted in a decrease in the production of lactic acid produced. The decrease in lactic acid production might increase the average pH value of the whey kefir seasoning since the total acid value was inversely proportional to the pH value. Thus, at high pH values, the total acid is considered low.

TABLE I. The Average Test Results of pH, Total Acidity and Protein Content

Treatment	pH	Total Acidity	Protein Content
P0	4.22 ± 0.01 [a]	1.21 ± 0.01 [d]	0.75 ± 0.01 [a]
P1	4.24 ± 0.01 [b]	1.15 ± 0.01 [c]	0.89 ± 0.04 [b]
P2	4.27 ± 0.02 [c]	1.10 ± 0.01 [b]	0.98 ± 0.04 [c]
P3	4.31 ± 0.03 [d]	1.08 ± 0.01 [b]	1.07 ± 0.02 [d]
P4	4.36 ± 0.01 [e]	1.02 ± 0.04 [a]	1.13 ± 0.01 [e]

Description: [a, b, c, d, e] Different superscripts in the same colum showed very significant differences ($P<0.01$) on pH, total acidity and protein content.

The increase in the average pH value due to the addition of sugar concentration also occurred in the study conducted by Gianti and Evanuarini (2011) that the increase in pH was caused by a concentrated sugar solution. Thus, the water in the cell would come out through the membrane and flow into the sugar solution. This event is called osmosis, which results in plasmolysis of microorganisms' cells, inhibiting their growth. In line with the above statement, Ariyanto, Hidayatulloh, and Murwono (2013) explained that giving sugar concentration treatment can inhibit the fermentation rate for it inhibits the growth rate of microbes that play a role in the fermentation process.

Total Acidity of Whey Kefir Seasoning

The analysis of variance showed that the addition of different concentrations of coconut sugar solution gave a very significant difference ($P<0.01$) to the total acid of whey kefir seasoning. The average value presented in Table I shows that the total acidity value of the whey kefir seasoning decreased along with the addition of the coconut sugar solution concentration. The highest average total acid value was found at P0 equal to 1.21 ± 0.01 without the addition of the coconut sugar solution concentration. Meanwhile, the lowest average total acidity value was found at P4, 1.02 ± 0.04, with the addition of the coconut sugar solution concentration of 50%.

The decrease in the average value of total acid of whey kefir seasoning was caused by the higher concentration of coconut sugar for it can cause a decrease in the activity of

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bacteria that played a role in the formation of lactic acid. The decrease in bacterial activity was due to the addition of the concentration of coconut sugar solution causing bacterial cells to lose water for their life activities. Since the additional concentration of coconut sugar solution made the water the food-bound, thus it cannot be used by bacteria. As a result, the growth of these bacteria was inhibited and there was a decrease in the production of lactic acid, which decreased the total acid value of the whey kefir seasoning. This is following the opinion of Tsakalidou and Papadimitriou (2011) which stated that the decrease in the total acid value is due to lactic acid bacteria that can experience osmotic stress at high sugar concentration. This is also supported by a statement made by Hartati, Pramono and Legowo (2012) that the treatment of adding sugar can inhibit the growth of Lactic Acid Bacteria (LAB), which inhibit the fermentation rate results in a decrease in the total acidity content of the product. This is due to the acidity of the product fermentation is influenced by the fermentation process which will convert lactose into lactic acid by lactic acid bacteria.

Protein Content on Whey Kefir Seasoning

The results of the analysis of variance showed that the addition of different concentrations of coconut sugar solution gave a very significant difference (P<0.01) to the protein content of whey kefir seasoning. The average value presented in Table I indicates that the average value of the protein content of the whey kefir seasoning increased with the addition of the concentration of coconut sugar solution. The lowest average value of protein content was at P0, 0.75 ± 0.01, without the addition of the coconut sugar solution concentration. Meanwhile, the highest pH average protein content was at P4, 1.13 ± 0.01, with the addition of a 50% coconut sugar solution concentration.

The increase in the average value of whey kefir seasoning protein content was due to the protein content of whey kefir seasoning determined by the quality of the basic raw materials used. The higher the protein content of the whey kefir, the higher the protein content in the resulting whey kefir seasoning. This is in line with the opinion of Herastuti, et al. (1994); Yusmarini and Efendi (2004) in Harun, Rahmayuni and Sitepu (2013) which state that the protein content contained in yogurt is the total amount of protein from the raw materials used and the protein of lactic acid bacteria contained therein. The protein content of bacteria ranged from 60-70%. In addition to the protein content derived from whey kefir, the addition of protein content also came from the addition of a higher concentration of coconut sugar solution. According to Wirastyo (2004) in Suwati, Ihromi and Asmawati (2019), coconut sugar contains a protein content of 14 g/100 g. Thus, the more the percentage of sugar is added, the protein content of whey kefir seasoning will increase. Moreover, sugar also contains several macronutrient and micronutrient elements estimated to have a higher content of both in coconut sugar compared to in white sugar, especially its protein content.

The Effect of Coconut Sugar Solution on Total Plate Count (TPC), Mold and Yeast on Whey Kefir Seasoning

The results of this study on the Total Plate Count (TPC), mold and yeast of the whey kefir seasoning without the addition of coconut sugar solution (P0), and whey kefir seasoning with the addition of coconut sugar solution 20% (P0), 30% (P2), 40% (P3) 50% (P4) are presented in Table II.

TABLE II. The Average Test Results of TPC, mold and yeast

Treatment	TPC	Mold and Yeast
P0	3.85 ± 0.01 ^{d}	2.40 ± 0.01 ^{a}
P1	3.81 ± 0.03 ^{d}	2.48 ± 0.02 ^{b}
P2	3.67 ± 0.01 ^{c}	2.59 ± 0.02 ^{c}
P3	3.58 ± 0.04 ^{b}	2.68 ± 0.02 ^{d}
P4	3.45 ± 0.04 ^{a}	2.76 ± 0.01 ^{e}

Description: $^{a, b, c, d, e}$ Different superscripts in the same colum showed very significant differences (P<0.01) on TPC, mold and yeast.

Total Plate Count (TPC) of Whey Kefir Seasoning

The analysis of variance showed that the addition of different concentrations of coconut sugar solution gave a very significant difference (P<0.01) to the TPC of whey kefir seasoning. The average value presented in Table 1 indicates that the average value of the Total Plate Count (TPC) of whey kefir seasoning decreased with the addition of the concentration of coconut sugar solution. The highest mean value of Total Plate Count (TPC) was at P0 which was 3.85 ± 0.01 without the addition of the coconut sugar solution concentration and the lowest Total Plate Count (TPC) value was found at P4 which was 3.45 ± 0.04 with the addition of the concentration of coconut sugar solution of 50%. The decrease in the average value of Total Plate Count (TPC) along with whey kefir was due to the addition of the concentration of coconut sugar solution which might inhibit the growth of microbial cells by reducing the water content of food to a minimum. Thus, the availability of water for microbial living activities becomes none and inhibits the growth of microbes that made these microbes unable to grow and develop for they need water for their life activities. Water played a role in the process of cell metabolism in liquid form. If the water was crystallized or bound in a coconut sugar solution, the water cannot be used by microbial cells. Coconut sugar solution can cause increased osmotic pressure on microbial cells since cell plasma water is absorbed by the solution outside the cell. This causes the cells to lack water which kills the microbes due to plasmolysis.

According to Fadimas, Rosyidi and Widati (2015), when a high concentration of sugar (40-50%) is added to food, it can inhibit microbial growth. This is also under the opinion of Safriani, Novita, Sulaiman and Ratino (2014) who explained that when sugar is added to food in high concentrations, it can cause some of the Aw (water activity) in food to decrease. Thus, it becomes unavailable for microbial living activities. Water activity (Aw) plays an important role in determining changes in microbial quality and survival because water activity (Aw) indicates the amount of free water available for microbial growth (Brown, 2011).

Mold and Yeast of Whey Kefir Seasoning

The results of the analysis of variance showed that the addition of different concentrations of coconut sugar solution gave a very significant difference (P<0.01) to the mold and

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yeast in whey kefir seasoning. The average value presented in Table 1 indicates the results of this study where the average value of mold and yeast for whey kefir seasoning increased with the addition of the concentration of coconut sugar solution. The lowest average value of mold and yeast was at P0, 2.40 ± 0.01 without the addition of the coconut sugar solution concentration, and the highest average value of mold and yeast was at P4, 2.76 ± 0.01 with the addition of coconut sugar solution concentration of 50%.

The increase in the average value of mold and yeast for whey kefir seasoning was due to the addition of sugar which can provide optimal conditions for mold and yeast growth. Since mold and yeast are resistant to extreme environmental conditions, mold and yeast can still survive in the high concentration of coconut sugar solution. This is following the statement from Widyastutik and Alami (2014); Hendritomo (2003) that mold and yeast can grow in a concentrated solution, such as in a solution of sugar and salt. The nature of molds and yeasts that are resistant to the environment (excess sugar, salt, and acidity) makes molds and yeasts able to survive and compete with other microorganisms.

The increasing number of yeast mold along with the increasing concentration of coconut sugar solution can also be caused by the fact that mold and yeast use the sugar as nutrients for their growth. The increase in the average value of yeast mold due to the addition of sugar concentration also occurred in the previous study conducted by Muchtar, Kamsina and Anova (2011) where sugar and its derivatives would be used by mold as a food source. Moreover, according to Meylani (2011), one of the microbes contained in sugar is yeast. This type of microbe is resistant to high sugar levels. Thus, it can ferment sugar into carbon dioxide and alcohol.

Best Treatment

Determination of the selection of whey kefir seasoning by adding the concentration of coconut sugar solution is best done using the collection effectiveness index method according to De Garmo, Sullivan and Canada (1984) using quantitative data parameters, namely pH, total acidity, protein content, Total Plate Count (TPC), mold and yeast. The product values can be seen in Table III.

TABLE III. The Value of Best Whey Kefir Seasoning Products

No.	Treatment	Total Value of Each Parameter
1.	P0	0.222
2.	P1	0.338
3.	P2	0.475
4.	P3	0.600
5.	P4	0.778

De Garmo, *et al.* (1984) stated that in determining the best treatment using the effectiveness index method, the value of the product will be obtained, where the treatment that has a high product value is better. In contrast, a treatment that has a low product value is the worse. Thus, the treatment that has the highest product value will be the best treatment. Based on these criteria, it was obtained whey kefir seasoning with the addition of coconut sugar solution concentration of 50% (P4) to be the best treatment with a product value of 0.778.

IV. Conclusion

The addition of the concentration of coconut sugar solution of 50% is the best treatment with a pH value of 4.36, total acidity of 1.02%, the protein content of 1.13%, Total Plate Count (TPC) of 3.45 log cfu/ml, mold and yeast of 2.76 log cfu/ml.

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Meilan Archadiya, Lilik Eka Radiati, and Manik Eirry Sawitri, “The Effect of Adding Coconut Sugar on Quality of Goat Milk Whey Kefir Seasoning from Physical, Chemical and Microbiological Qualities,” *International Research Journal of Advanced Engineering and Science*, Volume 6, Issue 1, pp. 39-43, 2021.